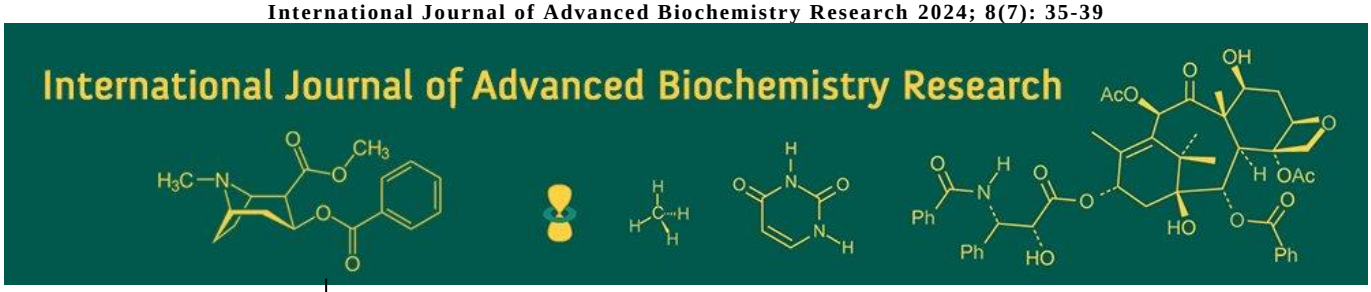

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Reduction in microbial load from the preputial cavity of black Bengal bucks after washing with Potassium permanganate solution

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Abstract
Quality semen production remains the main aim of semen processing laboratories. The bacteria most responsible for semen contamination originate from the preputial cavity and urinary tract of bucks that find their way through natural mating or artificially by semen collection. The main aim of the study is to determine the bacterial load in the Preputial cavity of Black Bengal Bucks before and after washing with 0.02% $KMnO_4$. There was a significant decrease (p<0.01) in bacterial load (39.6%), *Staphylococci* sp. (23%) and Coliform (25%). After preputial washing mean values (log CFU/ml) of Total plate count, *Staphylococci* spp count, the Coliform count were reduced to 3.77, 5.12, 1.45 from the initial value of 6.25, 6.70, 1.95, respectively. Hence, 0.02 % $KMnO_4$ solution can be used to wash the prepuce before routine semen collection from Bucks.

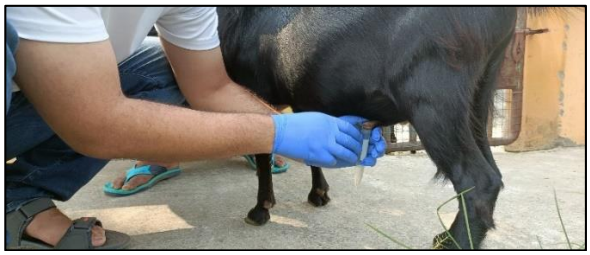
Keywords: Black Bengal goat, bacterial load, preputial washing, $KMnO_4$

Introduction
The success of an AI program depends largely on the production of quality sperm and appropriate AI processes (Patel *et al.* 2011) [18]. Semen quality is regarded as a measure of fertility in male animals. The major factors affecting semen quality include age, breed, genetics, nutrition, management, temperature, season scrotal circumference, etc. Besides these factors, a microbial load of the semen has a profound effect on the semen quality. One of the key factors, influencing sperm quality and further reproduction is the bacterial load in the preputial cavity (Griveau *et al*. 1995 and Diemer *et al.* 1996) [9, 7]. The preputial cavity is probably the most important source of bacteria that lead to reproductive diseases and the risk of microbial spread during sperm collection and subsequently used in artificial insemination. Though antibiotics can be used, as Prasad and Pachauri (1985) [19] used four different antimicrobial solutions (Benzylpenicillin and/or Streptomycin, Oxytetracycline) for preputial washing just before the collection of semen, which led to a decrease in the number of bacteria by 61% to 77% in semen. But, continuous use of antimicrobials may lead to bacterial resistance. Preputial washing with 0.02% $KMnO_4$ significantly reduces the bacterial load in Murrah bulls (Meena *et al.* 2015) [15].
In general, the process of collecting sperm is far from being a sterile process due to the involvement of many sources that can lead to bacterial contamination (Bussalleu and Torner, 2013) [5]. In this sense, additional measures such as routine animal and sperm monitoring, preputial washing, biosafety measures to reduce contamination during collection, processing, and storage, and sperm treatment with appropriate antimicrobials (Maes *et al.* 2008) [13] are required. Preputial washing is a managemental practice that is highly valuable for harvesting quality sperm by reducing bacterial load in the semen.
The present study aimed to enumerate the bacterial load in the preputial cavity of black Bengal bucks before and after washing with 0.2% $KMnO_4$ solution.
The present study was carried out at ICAR- National Dairy Research Institute (NDRI), Eastern Regional Station, Kalyani, West Bengal, India. Kalyani is located in the lower Gangetic basin of West Bengal in the Nadia district. Kalyani is situated at 22° 58'30"N latitude and 88° 26' 4" E longitude. The climatic condition is hot and humid. The average

annual maximum temperature is 39 °C and the minimum temperature is 12 °C. The maximum humidity is 91% and the minimum humidity is 58%. The annual rainfall is around 1250 mm.

Experimental Animals and their Management Black-Bengal (*Capra hircus bengalensis*) bucks (n=10) of 1.5 to 3.5 years of age, donating semen routinely were used in the study. The Bucks were provided with a concentrated mixture, mixed green grass, and *ad libitum* drinking water. Routine vaccination against *Peste des petits ruminant* (PPR), Goat pox, Enterotoxaemia, Hemorrhagic septicemia, Foot and mouth disease (FMD), and deworming were given.

Collection of Preputial Wash Before using the $KMnO_4$ solution, the prepuce was washed with 20 ml warm normal saline solution and collected in a sterile vial. Subsequently, 10 ml 0.2% $KMnO_4$ solution was used to wash the prepuce with the help of a sterile disposable plastic syringe. Washings of prepuce were collected in a sterile vial. Finally, preputial flushing samples were transferred to the laboratory for microbial estimation of Total plate count, Staphylococcus, and Coliform load.



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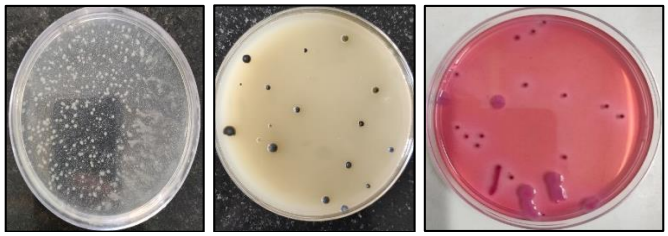
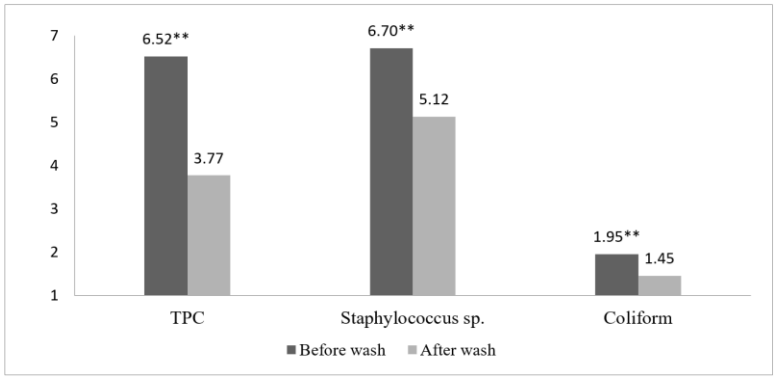


B C D

A; collection of preputial wash: B; Total plate count; C: *Staphylococci*; D: Coliform.

Estimation of Bacterial Load by Pour Plate Method; Samples of preputial wash were collected before and after washing with 0.02% $KMnO_4$ and were subjected to standard plate count by pour plate technique as per. For each sample serial dilution of 10^1 to 10^9 was carried out by using sterile 0.9% Normal saline. For microbial load count, (Plate count agar, Himedia®), coliform count (VRBA agar, Himedia®) and for Staphylococcus sp. (Baird parker, tellurite egg emulsion media, Himedia®) were used. All three agars were weighed and reconstituted in triple distilled water and then subjected to an autoclave at 121 °C for 30 minutes. One ml from each diluted sample was added to Petri plates and then uniformly mixed with respective agar. After mixing, plates were allowed to solidify and then incubated at 37ºC for 48 hrs. Serial dilution and plating were carried out under controlled laminar airflow. Plates were observed after 24-48 hrs and colonies were counted and expressed as CFU/ml. The data was first subjected to log transformation and then statistical analysis was carried out by one-way ANOVA using SPSS (Ver.20) and the significance was determined at $p<0.01$ level.

Before using the $KMnO_4$ solution, the prepuce was contaminated with a significantly ($p<0.01$) higher concentration (log CFU/ml) of Total Plate Count (6.52±0.06), *Staphylococci sp*. (6.70±0.06) and *E. coli* (1.95±0.05) respectively (Fig. 1). After washing with 0.02% $KMnO_4$ solution, the concentration of Total Plate Count, *Staphylococci sp*. and *E. coli* were reduced to 3.77±0.08, 5.12±0.14 and 1.45±0.08 (log CFU/ml) respectively. The reduction was by 42.1% (Total Plate Count), 23% (*Staphylococci sp*.) and 25% (*E. coli*), respectively (Fig. 2).



(Significance **$p<0.01$)

Fig 1: Bacterial load in the prepuce of Black Bengal Bucks before and after washing with 0.02% $KMnO_4$ solution

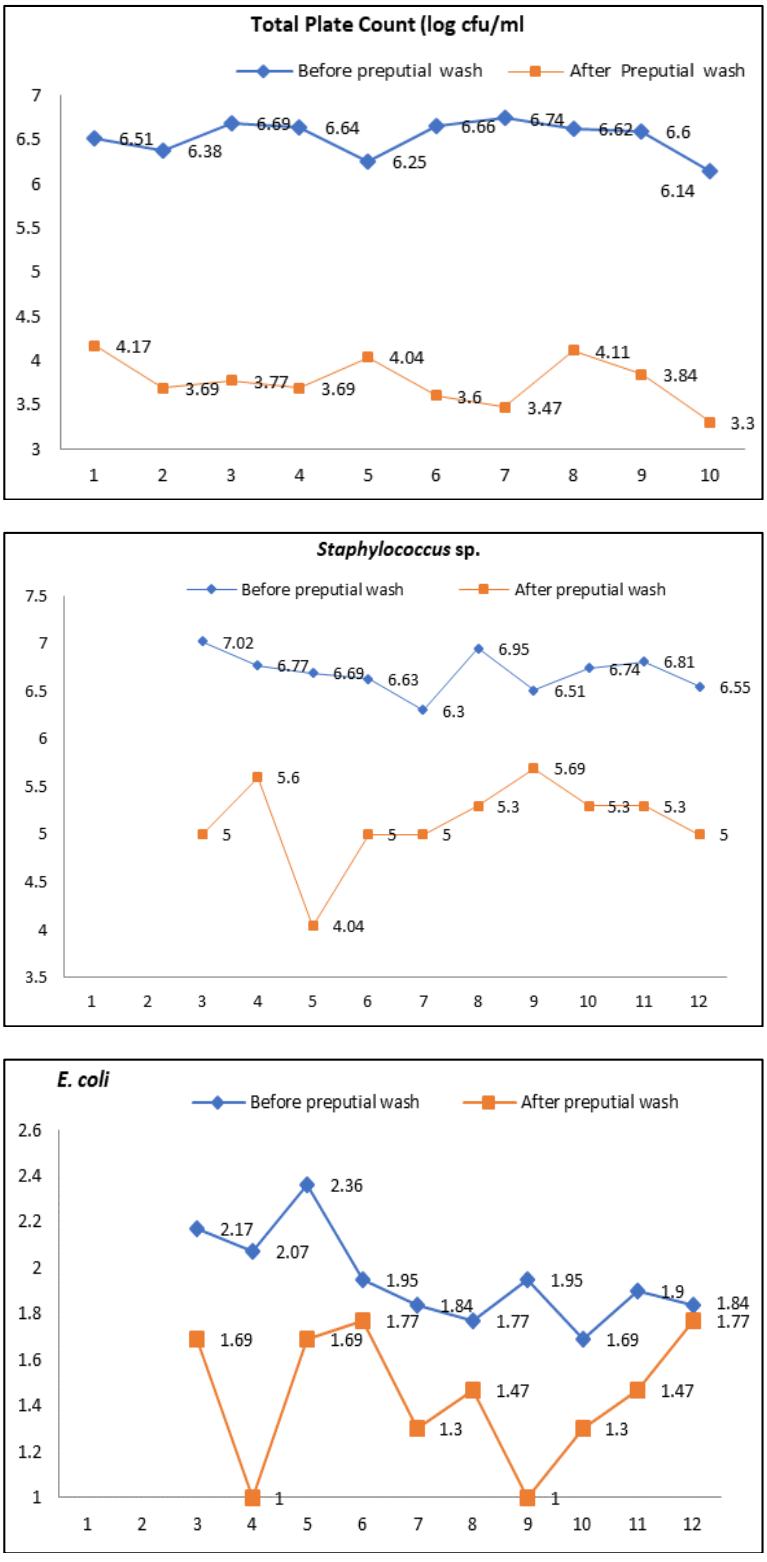


Fig 2: Representation of microbial load of prepuce before and after washing with $KMnO_4$

In case of bucks, scanty literature is available on preputial washing and bacterial load in the preputial cavity therefore, the discussion is supported using published literature on Bull cattle and Buffaloes. Meena *et al.* (2015) [15] found that preputial washing with 0.02% $KMnO_4$ solution would facilitate quality semen production in terms of reduced microbial load. They found the mean bacterial load of 3187.17, 2536.33 and 2292.83 (CFU/mlx10^3) in preputial washing by saline, savlon and $KMnO_4$ respectively. Ahmed *et al.* (2001) [3] in a study on Murrah bulls found that the bacterial count per ml was $253.05\times10^3\pm37.10\times10^3$, $14.70\times10^3\pm2.50\times10^3$, in preputial Washings and fresh semen, respectively.

The preputial cavity has a significant contribution to the microflora usually reported in semen. It may be due to normal microflora of the preputial cavity or due to the contact of the prepuce with the contaminated floor and other external environmental factors (Jansen and Wool-Board, 1983) [10]. Also because of the anatomical structure of the preputial sac, it has been found to harbor saprophytic microflora and other pathogens. Saprophytic microflora of the prepuce in healthy semen donors comprises numerous

bacterial species that may become associated with semen at ejaculation and during collection (Navya, 2012) [17]. Many studies confirm the presence of the same microbial species in prepuce as and in the semen, suggesting that the preputial cavity must be contributing majorly to the microbial load of semen. The current study has found a higher concentration of *Staphylococci* spp. and *Coliform* microorganisms in preputial washing samples. Different bacterial species have been isolated from ram prepuces such as *Streptococcus, Brucella abortus, Proteus mirabilis and Staphylococcus aureus* (Zaid and AL-Zubaidy, 2009) [27]. *Staphylococci* spp. is the most common member of normal microflora of sheep skin and that may be the reason for a high ratio of isolated *Staphylococcus aureus* from the prepuce in Rams. Corona *et al.* (2009) [6] found that semen samples were most frequently contaminated with *Staphylococci*, coliform, streptococci, etc. which negatively affect the motility and viability of bovine semen. Shallali *et al.* (2001) [22] also found that *Staphylococci aureus* was isolated in a higher ratio than other bacteria from the vagina of the healthy ewes which may be due to the transmission of these bacteria through natural services. Bacteria mainly reported in the semen of animals include *Coliforms, Corynebacterial, Micrococci, Proteus* spp, *Bacillus* spp, etc. Many other microorganisms have been isolated comparatively at lower frequencies and may be due to contamination from bedding, soil, air, manure and other environmental factors. These include species of *Staphylococci, Streptococci, Pseudomonas, Enterococci, Klebsiella, Yeasts,* etc. Although most of the bacteria that contaminate the preputial cavity are nonpathogenic, under suitable environmental conditions some of these bacteria may behave as opportunistic pathogens and may pose a significant risk to inseminated females like vaginitis, cervicitis, etc (Wierzbowski, 1981) [25].

Summary

The present study illustrated bacterial load in the preputial cavity of Black Bengal goats and a reduction in bacterial load of the preputial cavity after washing with 0.02% $KMnO_4$. *Staphylococci* spp. was the main organism followed by *Coliform* bacteria that occur in the preputial cavity of black Bengal goats. The presence of these pathogenic bacteria may decrease the fertility rate of artificial insemination and may also lead to the spread of infections from Buck to Doe during natural service**.** Therefore, it is necessary to have some managemental practice to reduce the bacterial load in the preputial cavity before semen collection or natural service.

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